

REMARKS

Applicants respectfully request cancellation of claims 1-3 and 36 without prejudice and without disclaimer as to the subject matter thereof. Thus, the claims in the case after entry of this amendment are claims 4-18 and 37.

35 U.S.C. §112, Second Paragraph

Applicants have amended claims 4 and 6 to include the step of incubating the lysate to allow the RNase to degrade the RNA molecules, as helpfully suggested by the Examiner. New claim 37, which is an independent form of cancelled claim 36, also includes this feature. Applicants, therefore, respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph, with regard to omitted steps.

Applicants have cancelled claim 36 and present new claim 37 in its place. Claim 37 essentially re-writes claim 36 in independent form, incorporating the limitations of independent claim 1. The claim specifies that the cells that produce the cellular component are the same cells that produce the RNase.

Claim 4 has been amended to recite that the cells that produce the cellular component are different cells than produce the RNase. Applicants earnestly submit that Claim 4 and Claim 37 are sufficiently distinct. As claim 1 has been canceled, and claims 4 and 37 are sufficiently distinct, Applicants respectfully request withdrawal of the objection under 37 C.F.R. §1.75(c).

Claim 7 has been amended, as helpfully suggested by the Examiner to include Markush language for the cellular component.

Applicants respectfully request withdrawal of the rejections under 35 U.S.C. §112, second paragraph.

35 U.S.C. §112, First Paragraph

The Examiner has rejected claims 15 and 17 under 35 U.S.C. §112, first paragraph. The Examiner reasons that RNase secreted into a space other than the periplasm would result in dilution

of the RNase such that "the RNase activity would be lost such that there would not be sufficient RNase activity to degrade substantially all of the RNA molecules present." (See Paper No. 16, page 3). Applicants disagree. We invite the Examiner's attention to the Specification at page 32, lines 9-28 through page 33, lines 1-4. In this passage the Specification teaches that RNase may be overexpressed and directed to the outside of the cell. The Specification cites four references in which RNase was produced and secreted using various signal peptides. Thus, contrary to the Examiner's assertion, the Specification provides the skilled artisan with sufficient guidance to enable production of RNase outside of the cell. The Specification teaches that RNase can be produced and secreted into the culture medium such that the RNase does not contact the cellular component *until the cells are lysed*. This reduces RNase toxicity and RNase degradation by proteolysis (see Specification page 32 lines 26-28). Thus, it is apparent from the teachings of the Specification that the RNase may be produced in the culture medium and be effective in degrading substantially all of the RNA upon lysis of the cells. Furthermore, one of skill in the art would know that the cells could not be grown in such copious amounts of medium as to completely dilute the RNase. Thus, the Examiner's concern is misplaced given the level of knowledge and skill in the art. The Examiner's blanket statement that any secreted RNase would be so diluted as to have insufficient RNase activity is not substantiated by any objective evidence, such as scientific journal articles or textbook citations. Thus, it appears that the Examiner is relying solely on information within his own personal knowledge, or is simply speculating. To the extent that the Examiner may be relying on information within his own personal knowledge, Applicants respectfully request an appropriate affidavit under 37 CFR §1.104(d)(2), or withdrawal of the rejection.

35 U.S.C. §102(b)

Turning to the merits of the Application, claims 1-5, 7-12, 14, 18, and 36 are clearly patentable over Meador and Kennell (1990) *Gene* 95:1-7 ("Meador"). Meador teaches the cloning and sequencing of the gene for RNase I. As described on page 3, last paragraph, the RNases were purified from the periplasm and spheroplasts of both overproducing strains (the generation of the

overproducing strains is described in the legend of Figure 2). The method of purification of the RNase from the overproducing strains is described in the last paragraph of page 3. Meador resuspended *spheroplasts* in buffer with vigorous agitation. Applicants respectfully point out to the Examiner that the cells were not lysed, as claimed in the instant application. The periplasm, or periplasmic space, is the region between the inner and outer membranes of a Gram-negative bacterial cell. Spheroplasts are formed when the outer membrane of the Gram-negative bacterium is partially removed. Thus, purification of enzymes from the periplasm by the formation of spheroplasts does not involve lysis of cells.

In Meador (page 3) the supernatant that resulted from the spheroplasts (essentially the periplasmic fraction) was dialyzed to degrade and eliminate any contaminating nucleic acid. This is completely different from lysing cells and demonstrating that the RNase present degraded substantially all of the RNA.

Generally, expression of RNase in the periplasm of bacteria and subsequent purification of RNase from the periplasm is performed while *minimizing cell lysis*. We invite the Examiner's attention to Meador *et al.* (1989) *Eur. J. Biochem.* 187:549-553 ("Meador II"), which was cited in the previous Office Action and subsequently withdrawn as a reference. Meador II clearly teaches the expression of RNase in the periplasm of bacteria and subsequent purification of RNase from the periplasm while *minimizing cell lysis* as release of DNA and other cellular components made subsequent purification of the enzyme difficult (see Meador II, page 549, column 2 under "Enzyme purification": "RNase I was purified from freshly grown bacteria, since some lysis occurred upon freeze-thawing to release DNA and other components that made subsequent purification difficult."). Thus, the Examiner's equating Meador's strategy with the invention as instantly claimed is simply incorrect. In sharp contrast to the teachings of Meador, an aspect of the Applicants invention is that, at the appropriate time, the cells are lysed to allow the periplasmic (or otherwise extracellular) RNase to access its intended intracellular RNA substrate. As Meador I and Meador II purified RNase from *spheroplasts*, Meador did not perform, teach or suggest that cellular components could be purified by lysis of cells containing cellular components and RNase such that the RNase could

degrade substantially all of the RNA in the cell lysate. Meador simply did not make a cell lysate in either Meador I or Meador II. Applicants respectfully request withdrawal of the rejection of claims under 35 U.S.C. § 102(b) over Meador.

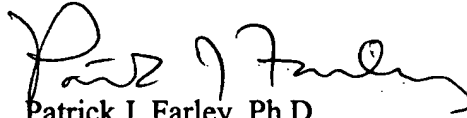
Claims 1-5, 7-11, 14, 18 and 36 have been rejected under 35 U.S.C. § 102(b) over Sambrook *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor, NY, 1989, pp. 1.38-1.39 ("Sambrook"). Nothing in this citation teaches or suggests that there is sufficient RNase to degrade substantially all of the RNA in the cell upon lysis using this protocol. Sambrook (page 1.39) does state that the precipitate that forms during storage at 0°C contains "high molecular weight RNA." Clearly this "high molecular weight RNA" is RNA that has not been degraded during the lysis procedure used in Sambrook. Elimination of this form of RNA not through the action of any RNase, but is accomplished in this protocol through centrifugation. Applicants respectfully submit that the claims are not anticipated by Sambrook and request withdrawal of the rejection of the claims under 35 U.S.C. § 102(b) over Sambrook.

Finally, the Examiner relies on his own statement that as enzymes (*e.g.* RNase) are catalysts "any reasonable amount of of RNase activity would be sufficient to degrade all of the RNA molecules present in the cell lysate." This assertion is unsupported by any reference to scientific literature, and appears to be the Examiner's own speculation. By this reasoning, one RNase molecule should degrade all of the RNA in the lysate, yet the Examiner speculates "any reasonable amount of RNase" should work, tacitly admitting that some amount would be necessary to degrade substantially all of the RNA. However, the Examiner proposes no amount of RNase that would be considered a "reasonable amount." Applicants respectfully request that the Examiner support this assertion with appropriate citations to the literature rather than rely on pure speculation.

Conclusion

In view of the foregoing, we request reconsideration, and urge prompt allowance of all the claims as amended.

Respectfully submitted,



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Version with Markings to Show Changes Made

Please cancel claims 1-3 without prejudice and without disclaimer as to the subject matter thereof.

4. (Twice Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing

(a) cells producing a cellular component; and

(b) cells producing an RNase[.];

lysing said cells producing said cellular component and said cells producing said RNase to produce a cell lysate, wherein said cells producing [an] said RNase produce said RNase in an amount sufficient to degrade substantially all of the RNA present in said cell lysate, incubating said lysate to allow RNase to digest said RNA molecules, and isolating said cellular component.

5. (Amended) The method of claim 4, wherein the cells producing the cellular component also produce [the] RNase.

6. (Twice Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing cells producing a cellular component and cells producing an RNase, wherein the cellular component and the RNase are not produced by the same cells, lysing said cells to produce a cell lysate, wherein said cells producing an RNase produce RNase in an amount sufficient to degrade substantially all of the RNA present in said cell lysate, incubating said lysate to allow RNase to digest said RNA molecules, and isolating said cellular component.

7. (Amended) The method of claim [1 or] 4, wherein said cellular component is selected from the group consisting of [one of] a recombinant DNA, a recombinant protein, and a recombinant

carbohydrate.

8. (Amended) The method of claim [1 or] 4, wherein said RNase is encoded by a gene that is integrated into the genome of the cell producing the RNase.
9. (Amended) The method of claim [1 or] 4, wherein said RNase is non-specific.
11. (Amended) The method of claim [1 or] 4, wherein said cell producing said RNase produces said RNase in a regulated manner.

Please cancel claim 36 without prejudice and without disclaimer as to the subject matter thereof.

Please add the following new claim:

37. (New) A method of preparing a substantially RNA-free cellular component, comprising culturing cells in a medium, wherein said cells produce said cellular component and RNase, and lysing said cells to produce a cell lysate, wherein said cell lysate contains said cellular component and RNase with sufficient RNase activity to degrade substantially all of the RNA molecules present in said cell lysate, incubating said lysate to allow RNase to digest said RNA molecules, and isolating said cellular component.